Detection of a novel ilarvirus in *Passiflora edulis* in Colombia

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Introduction

Colombia is one of the world's main producers and exporters of tropical fruits, however, it lacks a robust preventive **management programme for the control of plant viruses[1].**

Recent Next Generation Sequencing (NGS) according to Massart et al. (2017)[2] revealed 57% identity to the movement protein (MP) of *Lilac ring mottle virus* (*Ilarvirus*), and 65% identity to the replicase of the *Tomato necrotic streak virus* (*Ilarvirus*) in Colombian purple passion fruit farms. The spread of this virus could mean drastic reduction of crop yields and major economic losses. For this purpose, molecular biological tools such as nucleic acid isolation, polymerase chain reaction (PCR), and NGS are being used to measure the frequency and distribution of this virus in Colombia, to characterise which symptoms are associated with it, and to identify the pathways for its transmission.

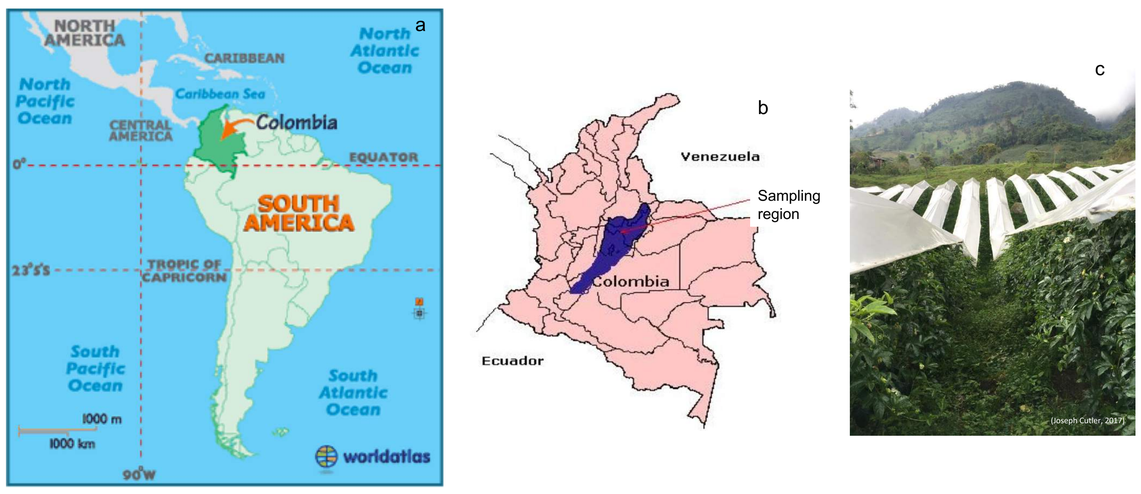

Figure 1. Maps of Colombia, where the new ilarvirus in purple passion fruit was found, and the region where the samples were taken from (a-b). Purple passionfruit field in Mesitas del Colegio, Colombia (c).

Materials and Methods

- Samples of *P. edulis* plant material with virus-suspected symptoms were collected in 2016 and 2017 from 5 different farms in the Altiplano Cundiboyasence Andean plateau (Cundinamarca and Boyaca departments, Colombia). 97 samples from these 5 farms, were used in this study.
- RNA isolation according to Boom et al. (1990)[3] from leaf material
- cDNA synthesis with pMMLV RTase (fresh material) or Premium RevertAid RTase (frozen material) and random hexamer primers
- nad5-PCR for quality control of RNA and cDNA synthesis according to Menzel et al. (2002)[4]
- Diagnostic RT-PCR for the detection of the novel ilarvirus with primers developed for its RNA1, RNA2, and RNA3
- RT-PCR for viral-specific detection of the CP coding region of RNA3 of the novel ilarvirus

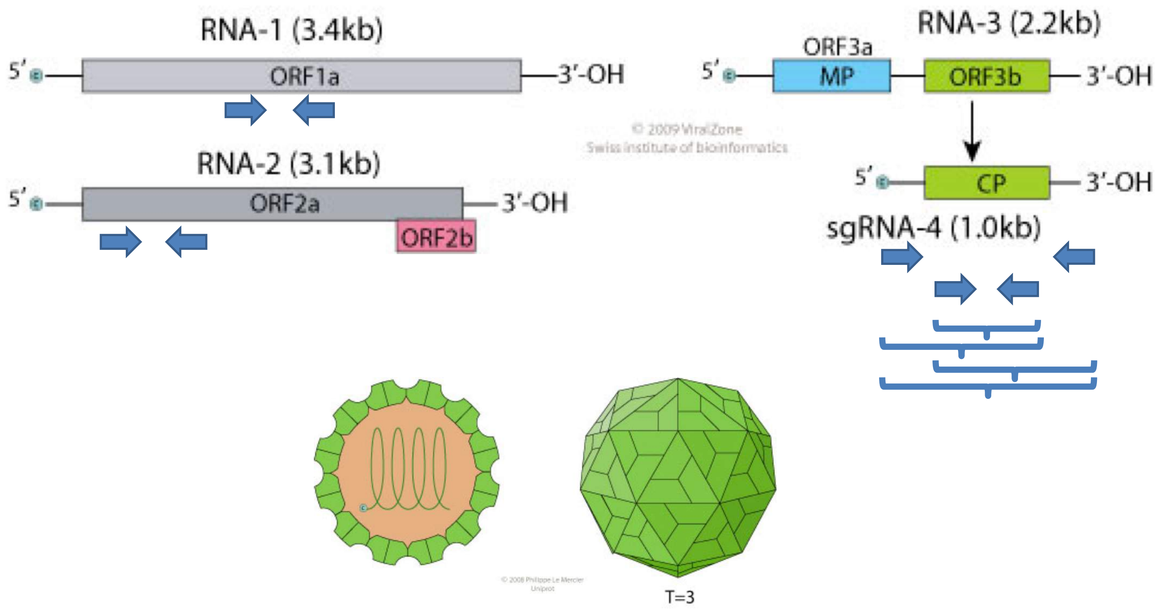

Figure 1. Genome structure of the *Ilarvirus* genus, with a tripartite linear ssRNA(+) genome. The positions of the developed primers are shown by blue arrows. For the RNA3 primers, the different combinations for amplification of the CP region by PCR are shown (a). Morphology of the ilarviruses (b). Figure adapted from ViralZone (2009).

Results

Figure 2. Healthy control and virus-suspected symptoms associated with the new ilarvirus tested by RT-PCR.

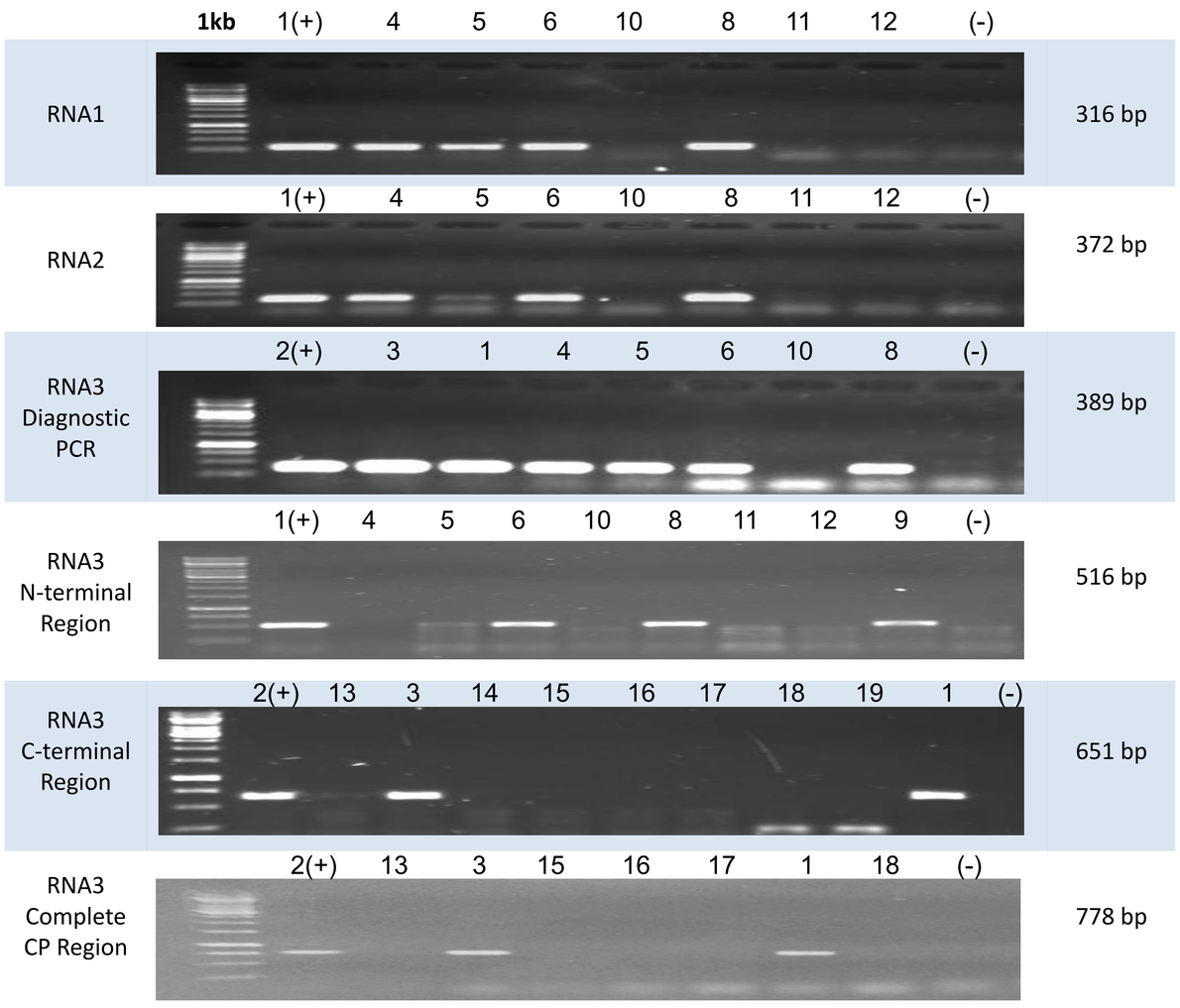

Figure 3. Detection of the new ilarvirus by means of RT-PCR, using designed primers based on the previously obtained NGS analysis. RNA1, RNA2 and RNA3 diagnostic PCRs, and primers targeting RNA3 N-terminal region, RNA3 C-terminal region, and RNA3 complete CP Region. In all PCRs, one positive control (+), previously tested, and water or a healthy control, were used as negative controls (-). Numbers from 1-19 refer to the same samples that were tested in the different PCRs.

Table 1. Results from the detection of the new ilarvirus by means of diagnostic RT-PCRs, using RNA1, RNA2, and RNA3 designed primers. Total number of samples processed: 97.

Symptom	Blistering	Yellowing	Chlorosis	Mottling	Blistering/ Deformation	Blistering/ Deformation/ Mottling	Blistering/ Mottling	Healthy
Samples with symptom	11	7	8	18	9	27	11	6
RNA1	1	0	0	1	4	2	1	0
RNA2	1	0	0	0	4	2	1	0
RNA3 Diagnostic	1	0	0	1	4	2	1	0
RNA3 N-terminal Region	1	0	0	1	4	2	1	0
RNA3 C-terminal Region	1	0	0	0	3	2	1	0
RNA3 Complete CP Region	1	0	0	0	3	1	0	0

Conclusions

The novel ilarvirus found in purple passion fruit (*Passiflora edulis* Sims) in Colombia can be detected by RT-PCR, using different types of primer combinations of the 3 RNAs of its genome. Therefore, this could be a useful tool to detect the presence of the virus in the passion fruit fields.

The virus could be detected in samples showing blistering, mottling, blistering/deformation, and blistering/deformation/mottling symptoms, as seen in Table 1. The coat protein (CP) gene is generally more variable. Therefore, the characterisation of the virus, is best done by amplification of the N-terminal and C-terminal regions of the CP region by separate RT-PCRs.

References

[1]Rodríguez, M. H., Niño, N. E., Cutler, J., Langer, J., Casierra, F., Miranda, D., Bandte, M., Büttner, C. (2016). Certificación de material vegetal sano en Colombia: un análisis crítico de oportunidades y retos para controlar enfermedades ocasionadas por virus. Revista Colombiana de Ciencias Hortícolas *10*(1), pp. 164-175.

[2]Massart, S., Candresse, T., Gil, J., Lacomme, C., Predajna, L., Ravnikar, M., Reynard, J., Rumbou, A., Saldarelli, P., Skoric, D., Vainio, E., Valkonen, J., Vanderschuren, H., Varveri, C., Wetzel, T. (2017). A Framework for the Evaluation of Biosecurity, Commercial, Regulatory, and Scientific Impacts of Plant Viruses and Viroids Identified by NGS Technologies. *Frontiers in Microbiology*, *8*, 45.

[3]Boom, R., Sol, C. J. A., Salimans, M. M. M., Jansen, C. L., Wertheim-van Dillen, P. M. E., van der Noordaa, J. (1990). Rapid and Simple Method for Purification of Nucleic Acids. Journal of Clinical Microbiology, 28 (3), pp. 495-503.

[4]Menzel, W., Jelkmann, W., Maiss, E. (2002). Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. Journal of Virological Methods, 99, pp. 81-92.

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